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Evolutionary history of polar and brown bears

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Abstract

Taxonomists have long recognized polar and brown bears as separate species with distinct ecological niches and largely nonoverlapping ranges. Surprisingly, phylogenetic studies of maternally inherited mitochondrial DNA (mtDNA) found polar bears nested within brown bears, with an estimated divergence time of <170,000 years. This indicated an unusually rapid speciation and adaptation of polar bears. However, several recent studies of autosomal and Y-chromosomal DNA have revisited these findings, giving independent perspectives of bear evolutionary history. Results show that polar bears cluster separately from brown bears, and divergence time estimates are older than those based on mtDNA, ranging from >300,000 to 4–5 million years. These studies confirm uniqueness of the polar bear lineage, provide more time for speciation and adaptation, and have uncovered numerous candidate genes for evolutionary adaptations. Several instances of introgressive hybridization between polar and brown bears have been inferred, revealing trans-species transmission of mtDNA and some nuclear loci.

Key words

Adaptation, Arctic, genome sequencing, introgressive hybridization, mtDNA, Pleistocene, speciation, *Ursus arctos*, *Ursus maritimus*, Y chromosome.

Key concepts

- DNA sequences in conjunction with a calibration of the mutation rate (e.g. inclusion of a previously estimated mutation rate, geological information, or inclusion of a radiocarbon-dated ancient sample) can be used to estimate the timing of speciation between species.
- Differentially inherited loci can reveal different aspects of evolutionary history.
- The genome of polar bears contains a wealth of alleles that are not found in brown bears, and vice versa.
- Polar bears have passed through bottlenecks during their evolutionary history, leaving them much less genetically variable than brown bears.
- When analyzing DNA sequences that have passed the species boundary due to introgressive hybridization, studies will obtain information about the hybridization event rather than the (earlier) speciation event.
- Brown bears have acted as vectors for polar bear alleles, transporting introgressed genetic material far beyond the species' contact zones.
- Incomplete lineage sorting (see glossary) complicates phylogenetic inferences among rapidly and/or recently diverged taxa. Lineage sorting takes on average $4 N_e$ generations, which for many taxa can span at least several hundred thousand years (N_e being the effective population size). The time needed for lineages to be reciprocally monophyletic can therefore take very long, even under complete reproductive isolation.
- Molecular studies have found signals of positive selection in polar bear genes that are involved in fat metabolism, energy production, and cardiovascular function. These genes are exciting candidates that may help us better understand the genetic basis of polar bear adaptations to Arctic conditions.

Introduction

Polar bears (*Ursus maritimus*) and brown bears (*Ursus arctos*) have long fascinated naturalists and the public, and bear ecology and evolution have been the focus of much research. Researchers in the mid to late 1900s recognized the vast morphological, physiological, and other differences between polar and brown bears, but also accepted that the two species had a relatively close evolutionary relationship. Since the early 1990s, studies of mitochondrial DNA (mtDNA) suggested a much closer relationship than expected between the two, showing (1) a paraphyletic relationship, with polar bears nested within brown bear diversity, and brown bears from the Alaskan Admiralty, Baranof, and Chichagof (ABC) Islands being more closely related to polar bears than to other brown bears, and (2) a surprisingly recent divergence of polar bears from their closest brown bear relatives, as suggested by tip-based phylogenetic dating methods (Drummond *et al.* 2002). Since 2011, however, several studies have revisited these findings utilizing a variety of approaches, complementing the mtDNA-based view of bear evolutionary history with analysis of other, independently inherited loci - and entire genomes - sequenced in multiple individuals per species. Here we review these new results in the light of previous findings, and discuss how this knowledge can be integrated to provide a better understanding of polar/brown bear evolution and adaptation.

A. TAXONOMY, SPECIATION AND INTROGRESSION

The view from classical taxonomy

Early modern taxonomists did not question that polar and brown bears are distinct species, given differences in morphology, behavior and distribution ranges (Fig. 1). Although the oldest polar bear fossil known until the late 1990s only dated back to the Late Pleistocene (a particularly large-bodied specimen found near Kew Bridge, London; Kurtén 1964), Kurtén (1964) estimated from allometric data that polar bears had diverged from brown bears in the Mid Pleistocene, a geological epoch spanning 781,000–126,000 years before present (YBP) (<http://quaternary.stratigraphy.org>). Kurtén proposed a scenario in which polar bears evolved from a Mid Pleistocene brown bear population that had become isolated in northern Arctic coastal regions, ultimately adapting to a highly carnivorous diet and life in Arctic sea ice habitats. This is consistent with newer findings from the fossil record, for example brown bear remains found in ca. 1.1 million year-old deposits in Europe (Wagner 2010). See also: DOI: 10.1002/9780470015902.a0001647.pub2, DOI: 10.1038/npg.els.0001635

Evidence from mtDNA and early studies of nuclear DNA

In the early 1990s, advances in DNA sequencing and phylogenetic analysis enabled a new look on polar/brown bear evolutionary history. Phylogenetic studies of mtDNA (Cronin *et al.* 1991; Talbot & Shields 1996; Waits *et al.* 1999) found polar bears nested within extant brown bear diversity, rendering brown bears paraphyletic (Fig. 2A). The mtDNA lineage of polar bears was consistently found to be most closely related to that of contemporary brown bears from the Alaskan ABC islands. This appeared consistent with Kurtén's (1964) allometry-based scenario of northern brown bear population isolation and subsequent evolution of the polar bear lineage.

Early studies of nuclear (i.e. non-mitochondrial) DNA (Yu *et al.* 2004; Pagès *et al.* 2008; Nakagome *et al.* 2008) recovered phylogenies which supported that polar and brown bears constitute sister species, but the studies included only a single representation per species and could thus not address the issue of paraphyly versus a sister lineage relationship:

when analyzing one sequence per species, one cannot assess whether one lineage is clustered within the diversity of another lineage. Hence, early nuclear genomic work constituted an important step towards resolving the bear phylogeny, but could not refute or confirm the branching pattern inferred from polar/brown bear mtDNA.

Discovery of a Pleistocene polar bear jawbone stimulates new work

In 2008, Ingólfsson & Wiig (2009) reported the discovery of a subfossil polar bear jawbone, found in a coastal sediment on the island of Svalbard (Barent's sea). Infrared stimulated luminescence dating suggested that the jawbone was ca. 80,000–150,000 years old, but further stratigraphic information suggested an age of 110,000–130,000 YBP. Stable isotope analysis indicated a marine diet, consistent with the current diet of polar bears rather than that of the terrestrial brown bear (Lindqvist *et al.* 2010). This finding was even more exciting because Lindqvist and colleagues also managed to sequence the entire mtDNA sequence of the individual – not only the oldest known polar bear fossil, but also the oldest mtDNA genome sequenced at the time. The jawbone's mtDNA clustered adjacent to, but just outside current polar bear variation. Using the estimated specimen age to obtain an improved dating of mtDNA branching events (see “Molecular Dating” in the glossary), Lindqvist *et al.* (2010) inferred that this ancient individual had died just some 20,000–30,000 years after the polar/brown bear mtDNA split, which was dated to ca. 150,000 YBP. Altogether, these findings indicated that the animal had died relatively shortly after the mtDNA divergence between extant brown and polar bears, and that its morphology and feeding behavior were already similar to that of current polar bears. This was surprising in the sense that such a short time scale for evolution of the distinct polar bear phenotype would render polar bears an example of unusually rapid speciation and adaptation among mammals.

Mitochondrial DNA from Irish bears further complicates the picture

Edwards *et al.* (2011) reported the sequencing of partial mtDNA control region sequences from Late Pleistocene/early Holocene bears from various locations on the British Isles. One surprising finding came from Irish bear remains, which based on morphology and stable isotopes appeared to originate from brown bears, and yet carried polar-bear like mtDNA. The authors proposed three scenarios which might potentially explain these findings, (1) evolutionary emergence of the polar bear lineage during the Late Pleistocene at ca. 45,000 YBP, with current polar bears deriving from descendants of this “Irish” stock of brown bears, (2) a slightly older but still Late Pleistocene speciation of polar bears, followed by mtDNA introgression (see glossary), or (3) a yet older speciation of polar bears during the Mid Pleistocene, followed by several instances of introgressive hybridization. Edwards *et al.* (2011) also analyzed a nuclear DNA data set which yielded a speciation time for polar/brown bears of 0.4–2 million YBP. However, this nuclear analysis was hampered by some of the same obstacles inherent in previously published analyses of DNA from the nuclear genome: besides limited resolution of the utilized nuclear markers, the analysis of only one representation per species precluded inferences regarding paraphyly or reciprocal monophyly (see glossary). The intriguing findings and well-crafted hypotheses of Edwards and colleagues furthered and stimulated the field, leading to some of the research presented below.

Statistically independent genetic evidence from the nuclear genome: multiple loci from multiple individuals per species

Analyzing sequences of 14 autosomal introns from the nuclear genome from 19 polar, 18 brown, 7 American black bears (*U. americanus*) and the giant panda (*Ailuropoda melanoleuca*) as an outgroup, Hailer *et al.* (2012) documented greater than expected levels of

unique nuclear genetic variation in polar bears. They inferred multilocus species trees (see glossary), in which polar and brown bears appeared as reciprocally monophyletic sister lineages (Fig. 2B). The species divergence was estimated to have occurred at ca. 338–940 thousand YBP (the 95% highest posterior density range), with a median at ca. 600 thousand YBP. Multilocus gene flow analyses did not find evidence of recent introgression among polar/brown bears, but alleles at some individual loci pointed towards signals of introgression in the nuclear genome, possibly resulting from past introgression events. The authors suggested that mtDNA paraphyly of brown bears might have resulted from Late Pleistocene introgression, with brown bear mtDNA being introgressed and subsequently replacing the original polar bear lineage – a process called mitochondrial capture. The study also found polar bears to have only ca. 20% of the nuclear variation in brown bears, consistent with past bottlenecks and/or long-term low effective population size. Given that many individual nuclear loci also show patterns of paraphyly (Nakagome *et al.* 2013) - especially when American black bears are included in the analysis (Hailer *et al.* 2013) - introgression and incomplete lineage sorting (see glossary, ILS) appeared to be impacting topologies at individual nuclear loci (Hailer *et al.* 2013). See also: DOI: 10.1002/9780470015902.a0025781

These processes of introgression and ILS become even more obvious when the entire bear family Ursidae is analyzed: based on data from autosomal introns and Y chromosome sequences, Kutschera *et al.* (2014) found evidence of introgressive hybridization among non-sister species of bears, with phylogenetic analysis of mitochondrial markers again recovering a significantly different topology than analyses of nuclear markers. Hence, introgression and incomplete lineage sorting have shaped the genomes of extant species into highly complex mosaic landscapes, where genomic regions differ from one another in terms of which evolutionary signals they portray, and many such linkage blocks commonly deviate from the overall species tree (Pollard *et al.* 2006; Jarvis *et al.* 2014; Campagna *et al.* 2015).

Despite these processes, autosomal sequences of brown and polar bears appear mostly differentiated from each other. A number of additional studies utilizing multilocus data from the nuclear genome have recently been conducted, all recovering a sister species relationship (monophyly) of polar and brown bears. Intron sequences, autosomal microsatellites, amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) markers analyzed in samples from across the ranges of polar and brown bears support the conclusion that the two species are not hybridizing extensively at present, and that their genomes are largely differentiated from each other (Hailer *et al.* 2012; Miller *et al.* 2012; Cronin & MacNeil 2012; Cronin *et al.* 2013, 2014; Liu *et al.* 2014). A notable exception are brown bears from the Alaskan ABC islands, in which prominent signals of introgression are found (see below).

Sequencing and evolutionary analysis of entire bear genomes

Miller *et al.* (2012) took bear genetics research to the genomics era, sequencing the full nuclear genomes of 23 contemporary polar bears, 3 brown bears (including 2 ABC bears) and one American black bear. Their phylogenetic analyses recovered the same overall species tree topology as found by Hailer *et al.* (2012). Based on a model that allows gene flow during the divergence process and assuming a mutation rate similar to humans, Miller *et al.* (2012) estimated the divergence of brown/polar bears to have occurred at ca. 4–5 million YBP, followed by a period of gene flow among the three bear species. Such introgression was particularly visible in the two brown bears from the ABC islands, which were estimated to contain ca. 5-12% polar bear alleles. In addition, Miller *et al.* (2012) found evidence of past population bottlenecks in polar bears (Fig. 3), and signals of adaptive evolution at numerous genes (for details, see the section below on adaptation).

Cahill *et al.* (2013) sequenced and analyzed the genomes of seven polar bears, one ABC brown bear, one brown bear from the Alaskan mainland, and an American black bear, along with genome data from Miller *et al.* (2012). By contrasting the observed variability levels for autosomal, X-linked and mtDNA markers, these studies revealed a pronounced sex bias in polar bear-derived alleles on the ABC islands, likely resulting from male brown bears immigrating into a Late Pleistocene polar bear population that had gotten isolated in this region. Male gene flow has subsequently diluted the original insular polar bear alleles, with higher swamping (lower percentage of polar bear alleles remaining) at loci that show a male-biased inheritance (autosomal > X > mtDNA) (Cahill *et al.* 2013). As subsequently shown by Cahill *et al.* (2015) who sequenced two additional ABC-Island and one European brown bear, brown bears have effectively acted as vectors for polar bear alleles (Hailer 2015): male-biased dispersal by brown bears has carried introgressed polar bear alleles far away from the original zone of admixture into brown bear populations resident on the adjacent North American mainland

Liu *et al.* (2014) reported the deep sequencing and *de novo* assembly of another polar bear genome. The authors used allele frequency and linkage information from 89 sequenced bear genomes for an in-depth demographic modelling of polar/brown bear evolutionary history [see next section for details on aspects of genomic adaptation signals]. The inferred model included an extended period of gene flow from polar into brown bears, and subsequent strong bottlenecks in polar bears. The inferred time since speciation was 343-479 ka, based on this model. Interestingly, the authors showed that inclusion of a strong post-speciation bottleneck in polar bears, evidenced by their other analyses, yields shorter times since speciation (i.e., a younger age of the polar bear lineage). This highlights that high-resolution data and complex models are necessary for reconstruction of detailed demographic/evolutionary scenarios, and that such scenarios may be needed for an accurate dating of past evolutionary events.

Y-chromosomal markers provide the male complement to the matrilineal view of polar/brown bear evolution

Bidon *et al.* (2014) characterized and genotyped 9 Y-linked microsatellite loci, and sequenced fragments totaling 5.3 kilobases on the Y chromosomes of 130 polar, brown and American black bears. Their analyses found polar and brown bears to be reciprocally monophyletic sister lineages - or “brother” lineages, given that this pattern was obtained from paternally and not maternally inherited loci (Fig. 2C).

It hence appears that little or no introgression is observed for the Y chromosome (Bidon *et al.* 2014), a pattern that however needs verification with larger sample sizes. The scenario of an “island conversion” by (Cahill *et al.* 2013, 2015) is thus supported by Y-chromosomal data, with apparent absence of Y chromosome introgression on the ABC islands (Bidon *et al.* 2014), suggesting that the original polar bear Y chromosome on these islands has been replaced by the Y chromosomes of immigrating brown bear males. This reinforces that differentially inherited loci portray different aspects of evolutionary history, especially in taxa with sex-biased dispersal and introgression.

Integrating multiple datasets to explain discordant mtDNA and nuclear phylogenies of brown and polar bears

The observed pattern of (1) mtDNA paraphyly of brown bears, contrasted by (2) autosomal multilocus species trees (despite introgression at some autosomal loci; see above) and Y-chromosomal phylogenies showing reciprocal monophyly of polar and brown bears, is still lacking a definite explanation (Edwards *et al.* 2011; Hailer 2015). There are at least three potential explanations for the overall pattern.

- (i) Perhaps the simplest explanation would be introgression of mtDNA from brown bears into polar bears (Edwards *et al.* 2011, Hailer *et al.* 2012, Miller *et al.* 2012). This would require hybrid mating of brown bear females with polar bear males. This suggestion conveniently addresses the surprising mtDNA similarity of brown bear populations as far apart as Western Europe and coastal Alaska: polar bears with their long distance dispersal and weak rangewide genetic structuring (Peacock *et al.* 2015) would have acted as a vector for introgressed brown bear mtDNA, transporting it across vast distances.
- (ii) Another explanation might be incomplete lineage sorting for mtDNA, but this seems intuitively surprising, given the low effective population size of polar bears that should favour mtDNA fixation in their lineage, and the fact that brown bear mtDNA shows the appreciated rangewide pronounced phylogeographic structuring (Davison *et al.* 2011).
- (iii) An intriguing explanation was recently provided by Hassanin (2015), who argued that all mtDNA lineage belonging to clade 1 (Fig. 2A; see Leonard *et al.* 2000 and Davison *et al.* 2011 for details about this nomenclature) should be designated as polar bear-derived. Under that hypothesis, not only the current polar bear lineages in clade 2b, but also the clades currently found in brown bear populations on the ABC islands (clade 2a) and Western Europe (clade 1) are ultimately derived from polar bears. Hassanin suggested that this occurrence in brown bears would derive from an introgressive hybridization event, where polar bear mtDNA entered the European brown bear gene pool (i.e., involving mating of a polar bear female with a brown bear male).

Analyses of additional extant bear genomes and especially of ancient bear remains may eventually answer this question. For example, it would be interesting to test whether the Irish bear remains with polar bear-like mtDNA (Edwards *et al.* 2011) show similar nuclear signals as the ABC-Island brown bears, which could support independent introgression events in coastal areas, shaped by male-biased immigration by brown bears into coastal habitats, analogous to the scenario inferred for the ABC islands (Cahill *et al.* 2013, 2015).

B. ADAPTATION IN POLAR AND BROWN BEARS

Since their divergence at least several hundred thousand years ago, polar and brown bears have evolved numerous morphological, ecological, and behavioral adaptations to cope with the distinctive features of their environments. Polar bears are the only bear species with an aquatic, strictly carnivorous lifestyle. Their skull is relatively longer and more flat than that of brown bears with the eyes set higher up. This may be helpful when swimming and hunting seals (Slater *et al.* 2010). The molars are also reduced, as in many other carnivores (Sacco & Van Valkenburgh 2004), but they lack a reinforced skull and the especially sharp teeth typically found in other species with a strictly carnivorous diet, which may be a consequence of their recent evolution from an omnivorous ancestor (Slater *et al.* 2010). The brown bear skull is better able to handle the stresses associated with grinding and chewing food, especially when using the molars (Slater *et al.* 2010). Polar bear diet is highly specialized on seal blubber (Stirling & McEwan 1975), which represents a high-energy food source. This allows polar bears to build up a thick layer of fat that provides energy reserves and to some extent insulation (Pond *et al.* 1992). Polar bears may also have different physiological adaptations to periods of fasting both within and outside the denning period (Derocher *et al.* 1990; Lennox & Goodship 2008). Similar to brown bears, polar bears have a

dense layer of underfur with interspersed guard hairs (DeMaster & Stirling 1981) to provide warmth, although in polar bears the fur lacks pigment, which aids in camouflage. Nevertheless, it is challenging to unequivocally demonstrate the adaptive benefits of polar and brown bear characteristics, because these animals are difficult to house and/or manipulate for experimental tests.

Molecular adaptations in polar bears

With the recent developments in sequencing technology it has become possible to explore the polar bear genome to investigate signatures of selection and identify candidate genes (see glossary) that may harbor adaptive differences. Signatures of selection in the genome have largely been restricted to genes related to energy production, fat metabolism, and cardiovascular function. Liu and colleagues (2014) found evidence of positive selection in the *APOB* gene, which produces apolipoprotein B (ApoB). The ApoB protein binds lipids, such as low-density lipoproteins (LDL), transports them in the blood, and aids in moving them into cells. In humans, LDL has been associated as a major risk factor for heart disease and other cardiovascular disorders. Liu et al (2014) also discovered evidence for positive selection in additional genes that play roles in cardiovascular function, suggesting that these genes contain polar bear adaptations for coping with a high fat diet, while avoiding impacts on the cardiovascular system. Similarly, Miller et al. (2012) identified several candidate genes in polar bears, discovering fixed substitutions predicted to alter the gene function, which might be associated with lipid metabolism. Miller et al. (2012) identified the *FTO* gene, known to be correlated with obesity in humans, and the *PLTP* gene, which is involved in synthesis and metabolism of high-density lipoproteins (HDL). The authors identified additional genes involved in long-chain fatty acid synthesis, and the *BTN1A1* gene that has been shown to influence fat content of milk. Welch *et al.* (2014) examined potential molecular adaptations in cellular respiration, the primary metabolic pathway involved in the production of cellular energy and heat. They identified substitutions in several genes that might regulate the concentration of nitric oxide, which could possibly control the trade-off between using food resources to produce energy for locomotion and hunting versus producing heat to maintain body temperatures in the extreme Arctic environment.

There has also been much interest in examining the genetic basis underlying one of the polar bear's most distinctive adaptations: its white fur. A variant of the *EDNRB* gene is apparently fixed in some polar bears, and this protein is associated with the migration of precursory pigment producing cells (Miller et al 2012). Furthermore, seven polar-bear specific substitutions were identified in the candidate gene *LYST* (Liu et al 2014), one of which occurred in a conserved domain and was predicted to cause functional change. Mutations in any of these genes could potentially reduce the production and/or transport of pigments into polar bear hairs. Interestingly, the mechanism leading to a lack of pigmentation in polar bear hair seems to differ from that in white phenotype black bears living on the northwest coast of British Columbia, and in other mammals (Ritland *et al.* 2001; Nachman *et al.* 2003).

Molecular adaptations in brown bears

The molecular basis for adaptations of brown bears have been less well characterized than for polar bears. In one case no significant evidence for selection was found (Liu et al 2014), although in another study (Welch et al 2014) evidence for selection was identified in the *IRS1* gene, which is related to insulin and glucose uptake from the blood. This was suggested as a potential adaptation for either dealing with the large amounts of carbohydrates consumed during hyperphagia prior to hibernation, or to switching energy sources between using carbohydrates when active versus relying on stored fat during hibernation.

Limitations and future prospects for understanding bear adaptation

We are in the earliest stages of understanding the genetic underpinnings of ecologically important traits. Detecting selection at the molecular level is difficult and determining the functional significance of changes remains challenging. Demography may influence levels of diversity, and polar bears likely experienced past population bottlenecks (Miller *et al.* 2012). Drift in small populations may also make selection less efficient. Further, conclusions rely on robust sampling, which has been especially limited for the geographically widespread and genetically diverse brown bear. Beyond these, computational predictions that substitutions alter the performance of a protein do not necessarily mean that they lead to beneficial changes, and most changes are thought to reduce protein function or be only weakly beneficial (Eyre-Walker & Keightley 2007). Despite these limitations, use of genome-scale resources has led to the identification of genes underlying important ecological traits in other species (e.g. Jones *et al.* 2012), and thus candidate loci identified in polar bears will likely yield important insights as well. Given the recent shared ancestry between polar and brown bears, many of the adaptive changes in these species may occur outside of protein coding regions, which have been the main focus of most work so far, but have relatively slow substitution rates. Changes occurring in regulatory regions may adaptively alter gene expression and activity without directly altering the structure of the protein product. This represents an exciting but challenging area of research (Hardison & Taylor 2012). Another avenue for future research will be to investigate candidate genes in more detail, including genotyping more individuals to understand the pattern of variants over the entire range (especially in brown bears), as well as exploring the impact of these variants on form and function.

C. OUTLOOK

In conclusion, polar bears are a genetically distinct lineage with numerous unique genetic adaptations. Likely having persisted for several hundred thousand years, polar bears as a species have experienced several glacial cycles. However, the polar bear genome carries testimony of past population bottlenecks that were presumably linked to environmental changes such as interglacial warm phases - indicative of vulnerability to climatic changes (Durner *et al.* 2009; Peacock *et al.* 2015; Kutschera *et al.* 2016). In addition, although polar bears have persisted through previous warm phases, multiple current human-mediated stressors (e.g., habitat conversion, persecution, and accumulation of toxic substances in the food chain) are magnifying the impacts of current climate change. Altogether, these factors are posing a novel and profound threat to polar bear survival.

Brown bears occupy a vast range across Europe, Asia and North America, with investigations of local adaptations being an exciting avenue for further research. Longer duration of the summer sea ice melt in coming years may leave the possibility open for polar and brown bears to come into increasing contact (Kelly *et al.* 2010), with polar bears spending more time on land, and brown bears expanding their range northward.

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Glossary

Candidate gene: A locus for which there is evidence that it may be related to a phenotype of interest. Generally knowledge of the function of the locus comes from studies of model organisms such as humans.

Incomplete lineage sorting (ILS): The process of genetic polymorphisms passing through speciation events and eventually sorting into separate diagnosable lineages.

Introgression: The process when part of the genome crosses species boundaries and enters the gene pool of another species through hybridization and subsequent backcrossing to one of the original species.

Molecular dating: Ages of past evolutionary events are inferred by using DNA sequences in conjunction with a calibration. This calibration identifies the time required for the accumulation of observed mutations, and is typically derived from the appearance of the organisms in the fossil record or by obtaining sequences from organisms from some known time point in the past.

Monophyly: An evolutionary relationship observed from a phylogenetic tree, in which all individuals of a species A are more closely related to each other than to members of any other species.

Paraphyly: A relationship observed in a phylogenetic tree in which some individuals of species A are more closely related to individuals of another species B, than they are to other members of their own species A.

Species tree: A phylogenetic tree built from multiple independent loci which takes into account that each locus may demonstrate a different evolutionary history, due to processes such as incomplete lineage sorting, introgression, and locus duplication.

Figures:

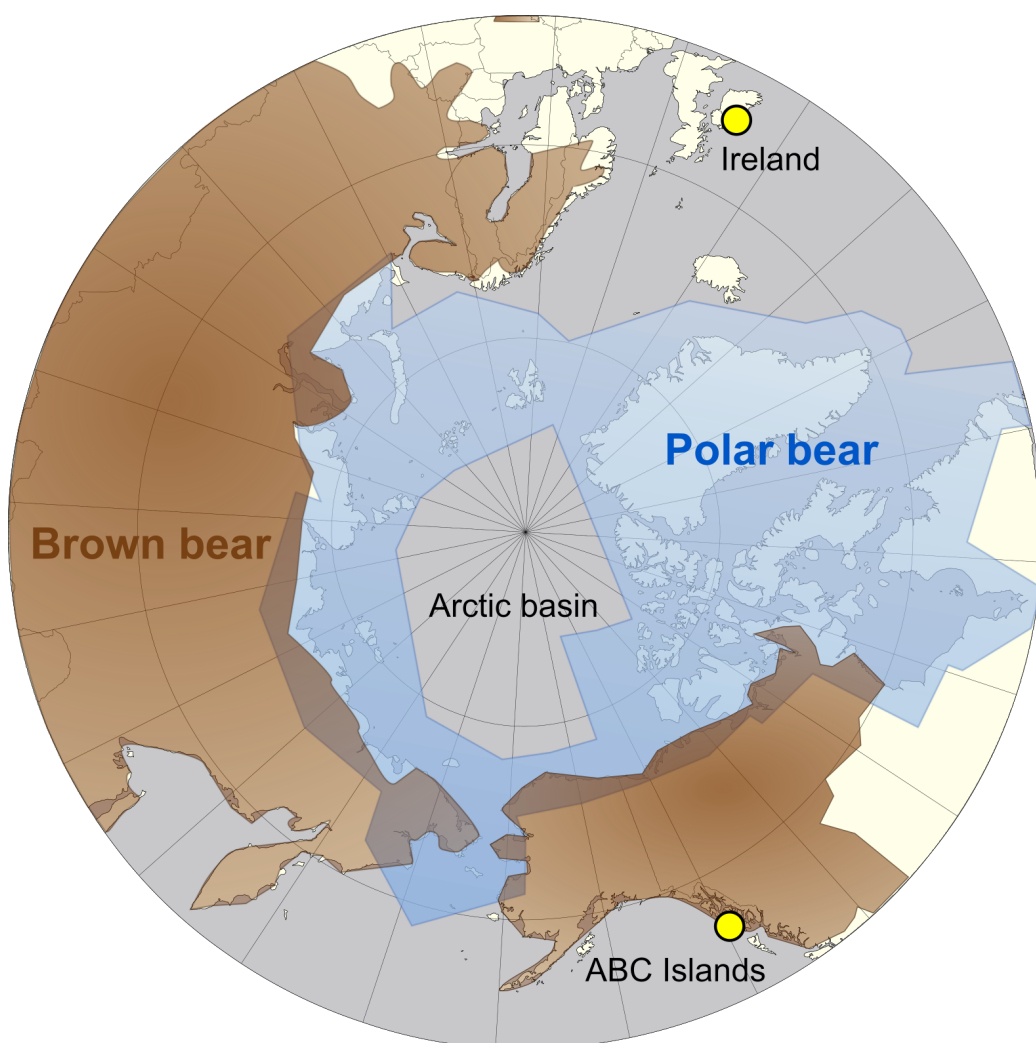


Fig. 1: Distribution ranges of polar and brown bears (shown in blue and brown, respectively). Yellow circles denote the Alaskan ABC Islands and Ireland, where genetic data indicate that introgressive hybridization has occurred in the past. Range information is taken from the IUCN and additional sources.

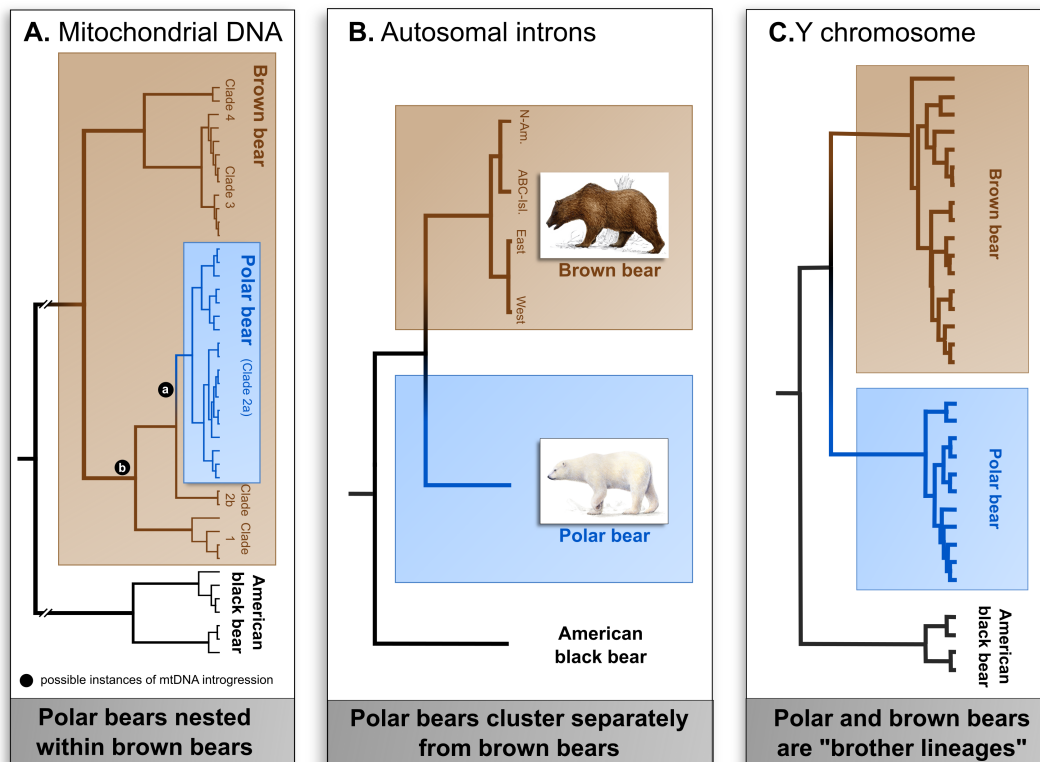


Fig. 2: Phylogenies of mtDNA, autosomes, Y chromosomal data from polar, brown, and American black bears. (A) Phylogeny of partial mtDNA control region data, showing paraphyly. (B) Species tree based on 14 autosomal intron markers, and (C) phylogeny of 5.2 kilobases of Y-chromosomal sequence data, both showing polar and brown bears as reciprocally monophyletic lineages. Black circles in (A) show previously suggested instances of past mtDNA introgression that may explain the incongruence between mtDNA and nuclear loci. Modified from Hailer et al. (2012) (A, B) and Bidon et al. (2014) (C). Bear illustrations: © Fauna, www.fauna.is.

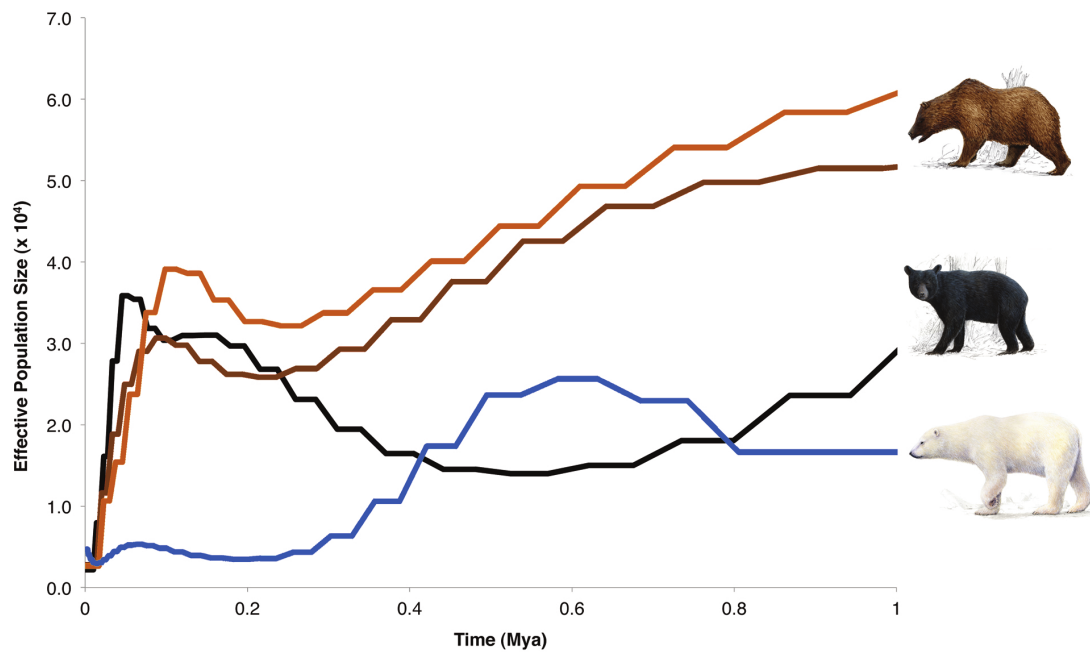


Fig. 3: Changes in effective population size through time estimated using PSMC (Li & Durbin 2011) from whole genome sequencing. Brown bears shown in brown (light brown: ABC Islands, dark brown: Alaskan mainland), black and polar bears are shown in black and blue, respectively. Time (shown in million years ago; Mya) was calibrated by assuming a mutation rate of 1×10^{-9} per site per year, similar to humans, and a generation time of 10 years. Modified from Miller et al. (2012). Bear illustrations: © Fauna, www.fauna.is.